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§Appl. No. 09/996,956
Amdt. dated September 1, 2004
Reply to Office Action of, April 1, 2004

Withdrawal of the restriction between PR33a and PR33b is requested. As explained in the response filed July 1, 2003, these sequences are highly related. A search for PR33a would necessarily overlap with PR33b.

For example, as explained in detail on Page 3-6 of the specification, SEQ ID NOS 1 and 3 are related sequences:

PR33a (SEQ ID NO. 1) and PR33b (SEQ ID NO. 3) are structurally related sequences. PR33a is about 5217 nucleotides in length, including a polyA tail, and has two Alu-type sequences at about nucleotide positions 319-440 (Alu I) and 2010-2226 (Alu II), both in a reverse or antisense orientation. PR33b is about 5093 nucleotides in length, including a polyA tail, and has a single Alu sequence in reverse at nucleotide positions 1837-2092 which corresponds to the Alu II sequence of PR33a, but is missing the Alu I sequence. SEQ ID NO. 2 is the nucleotide sequence which is present in PR33a, but absent from PR33b. PR33a has an additional CAG triplet (the Alu I sequence, itself, has a 3' CAG triplet at its terminus) adjoining the 3' end of its Alu I sequence which is absent from PR33b. Other than these two differences, PR33a and PR33b share the same nucleotides sequence and appear to arise from the same gene (see below). In addition to the transcripts corresponding to PR33a and PR33b, other cDNAs arising from the same gene have been detected. These are described in more detail below in the section describing genomic DNA.

Thus, it is not logical to restrict the claims between these two nucleotide sequences.

The claims stand rejected over 35 U.S.C. §101. Attached is a post-filing publication (Exhibit B) establishing that expression of PCGEM1, a shorter and incomplete form of the PR33 family (sequences are compared in Exhibit A), is significantly higher in prostate cancer. This publication provides clear evidence of a utility of PR33, as asserted in the specification, e.g., Page 1, lines 15-30.

In view of the above remarks, favorable reconsideration is courteously requested. If there are any remaining issues which could be expedited by a telephone conference, the Examiner is courteously invited to telephone counsel at the number indicated below.

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The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,



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Attorney Docket No.: ORIGEN-0034

Date: September 1, 2004

PR33a	GAAACTTTAAATATCCCTCAGTGCTCCTGTTAATTCATGGTAGTGCCCCAAGGCACTCT
PCGEM1	-----AAGGCACTCT
PR33b	GAAACTTTAAATATCCCTCAGTGCTCCTGTTAATTCATGGTAGTGCCCCAAGGCACTCT

PR33a	GGCACCCAGTTTTGGAAGTGCAGTTTTAAAAGTCATAAATTGAATGAAAATGATAGCAAA
PCGEM1	GGCACCCAGTTTTGGAAGTGCAGTTTTAAAAGTCATAAATTGAATGAAAATGATAGCAAA
PR33b	GGCACCCAGTTTTGGAAGTGCAGTTTTAAAAGTCATAAATTGAATGAAAATGATAGCAAA

PR33a	GGTGGAGGTTTTTAAAGAGCTATTTATAGGTCCCTGGACAGCATCTTTTTTCAATTAGGC
PCGEM1	GGTGGAGGTTTTTAAAGAGCTATTTATAGGTCCCTGGACAGCATCTTTTTTCAATTAGGC
PR33b	GGTGGAGGTTTTTAAAGAGCTATTTATAGGTCCCTGGACAGCATCTTTTTTCAATTAGGC

PR33a	AGCAACCTTTTTTGCCCTATGCCGTAACCTGTGTCTGCAACTTCCTCTAATTGGGAAATAG
PCGEM1	AGCAACCTTTTTTGCCCTATGCCGTAACCTGTGTCTGCAACTTCCTCTAATTGGGAAATAG
PR33b	AGCAACCTTTTTTGCCCTATGCCGTAACCTGTGTCTGCAACTTCCTCTAATTGGGAAATAG

PR33a	TTAAGCAGATTCATAGAGCTGAATGATAAAATTGTACTACGAGATGCACTGGGACTCAAC
PCGEM1	TTAAGCAGATTCATAGAGCTGAATGATAAAATTGTACTACGAGATGCACTGGGACTCAAC
PR33b	TTAAGCAGATTCATAGAGCTGAATGATAAAATTGTACTACGAGATGCACTGGGACTCAAC

PR33a	GTGACCTTATCAAGTGAGATGGAGTCTTGCCCTGTCTCCAAGGCTGGAGCCCAATGGTGT
PCGEM1	GTGACCTTATCAAGTGAG-----
PR33b	GTGACCTTATCAAGTGAG-----

PR33a	GATCTTGGCTCACTGCAACCTCCACCTCCAGGTTCAAACGTTTCTCCTGCCTCAGCCTC
PCGEM1	-----
PR33b	-----
PR33a	CCAAGTAACTGGGATTACAGCAGGCTTGGTGCATTTGACACTTCATGATATCAGCCAAAG
PCGEM1	-----CAGGCTTGGTGCATTTGACACTTCATGATATCAGCCAAAG
PR33b	-----GCTTGGTGCATTTGACACTTCATGATATCAGCCAAAG

PR33a	TGGAACATAAAACAGCTCCTGGAAGAGGACTATGACATCATCAGGTTGGGAGTCTCCAGG
PCGEM1	TGGAACATAAAACAGCTCCTGGAAGAGGACTATGACATCATCAGGTTGGGAGTCTCCAGG
PR33b	TGGAACATAAAACAGCTCCTGGAAGAGGACTATGACATCATCAGGTTGGGAGTCTCCAGG

PR33a	GACAGCGGACCCTTTGGAAAAGGACTAGAAAAGTGTGAAATCTATTAGTCTTCGATATGAA
PCGEM1	GACAGCGGACCCTTTGGAAAAGGACTAGAAAAGTGTGAAATCTATTAGTCTTCGATATGAA
PR33b	GACAGCGGACCCTTTGGAAAAGGACTAGAAAAGTGTGAAATCTATTAGTCTTCGATATGAA

PR33a	ATTCTCTGTCTCTGTAAAAGCATTTTCATATTTACAAGACACAGGCCTACTCCTAGGGCAG
PCGEM1	ATTCTCTGTCTCTGTAAAAGCATTTTCATATTTACAAGACACAGGCCTACTCCTAGGGCAG
PR33b	ATTCTCTGTCTCTGTAAAAGCATTTTCATATTTACAAGACACAGGCCTACTCCTAGGGCAG

PR33a	CAAAAAGTGGCAACAGGCAAGCAGAGGGAAAAGAGATCATGAGGCATTTTCAGAGTGCACT
PCGEM1	CAAAAAGTGGCAACAGGCAAGCAGAGGGAAAAGAGATCATGAGGCATTTTCAGAGTGCACT

PR33b	CAAAAAGTGGCAACAGGCAAGCAGAGGGAAAAGAGATCATGAGGCATTTTCAGAGTGCACCT *****
PR33a PCGEM1 PR33b	GTCTTTTCATATATTTCTCAATGCCGTATGTTTGGTTTTATTTTGGCCAAGCATAACAAT GTCTTTTCATATATTTCTCAATGCCGTATGTTTGGTTTTATTTTGGCCAAGCATAACAAT GTCTTTTCATATATTTCTCAATGCCGTATGTTTGGTTTTATTTTGGCCAAGCATAACAAT *****
PR33a PCGEM1 PR33b	CTGCTCAAGAAAAAAAAATCTGGAGAAAACAAAGGTGCCTTTTGCCAATGTTATGTTTCTT CTGCTCAAGAAAAAAAAATCTGGAGAAAACAAAGGTGCCTTTTGCCAATGTTATGTTTCTT CTGCTCAAGAAAAAAAAATCTGGAGAAAACAAAGGTGCCTTTTGCCAATGTTATGTTTCTT *****
PR33a PCGEM1 PR33b	TTTGACAAGCCCTGAGATTTCTGAGGGGAATTCACATAAATGGGATCAGGTCATTCATTT TTTGACAAGCCCTGAGATTTCTGAGGGGAATTCACATAAATGGGATCAGGTCATTCATTT TTTGACAAGCCCTGAGATTTCTGAGGGGAATTCACATAAATGGGATCAGGTCATTCATTT *****
PR33a PCGEM1 PR33b	ACGTTGTGTGCAAATATGATTTAAAGATACAACCTTTGCAGAGAGCATGCTTTCTTAAGG ACGTTGTGTGCAAATATGATTTAAAGATACAACCTTTGCAGAGAGCATGCTTTCTTAAGG ACGTTGTGTGCAAATATGATTTAAAGATACAACCTTTGCAGAGAGCATGCTTTCTTAAGG *****
PR33a PCGEM1 PR33b	GTAGGCACGTGGAGGACTAAGGGTAAAGCATTCCTCAAGATCAGTTAATCAAGAAAGGTG GTAGGCACGTGGAGGACTAAGGGTAAAGCATTCCTCAAGATCAGTTAATCAAGAAAGGTG GTAGGCACGTGGAGGACTAAGGGTAAAGCATTCCTCAAGATCAGTTAATCAAGAAAGGTG *****
PR33a PCGEM1 PR33b	CTCTTTGCATTCTGAAATGCCCTTGTTGCAAAATATTGGTTATATTGATTAAATTTACACT CTCTTTGCATTCTGAAATGCCCTTGTTGCAAAATATTGGTTATATTGATTAAATTTACACT CTCTTTGCATTCTGAAATGCCCTTGTTGCAAAATATTGGTTATATTGATTAAATTTACACT *****
PR33a PCGEM1 PR33b	TAATGGAAACAACCTTTAACTTACAGATGAACAAACCCACAAAAGCAAAAAATCAAAAGC TAATGGAAACAACCTTTAACTTACAGATGAACAAACCCACAAAAGCAAAAAATCAAAAGC TAATGGAAACAACCTTTAACTTACAGATGAACAAACCCACAAAAGCAAAAAATCAAAAGC *****
PR33a PCGEM1 PR33b	CCTACCTATGATTTTCATATTTTCTGTGTAACTGGATTAAAGGATTCTTGCTTGCTTTTGG CCTACCTATGATTTTCATATTTTCTGTGTAACTGGATTAAAGGATTCTTGCTTGCTTTTGG CCTACCTATGATTTTCATATTTTCTGTGTAACTGGATTAAAGGATTCTTGCTTGCTTTTGG *****
PR33a PCGEM1 PR33b	GCATAAATGATAATGGAATATTTCCAGGTATTGTTTAAATGAGGGCCCATCTACAAATT GCATAAATGATAATGGAATATTTCCAGGTATTGTTTAAATGAGGGCCCATCTACAAATT GCATAAATGATAATGGAATATTTCCAGGTATTGTTTAAATGAGGGCCCATCTACAAATT *****
PR33a PCGEM1 PR33b	CTTAGCAATACTTTGGATAATTCTAAAATTCTAGCTGGACATTGTCTAATTGTTTTTTATA CTTAGCAATACTTTGGATAATTCTAAAATTCTAGCTGGACATTGTCTAATTGTTTTTTATA CTTAGCAATACTTTGGATAATTCTAAAATTCTAGCTGGACATTGTCTAATTGTTTTTTATA *****
PR33a PCGEM1 PR33b	TACATCTTTGCTAGAAATTTCAAATTTTAAGTATGTGAATTTAGTTAATTAGCTGTGCTGA TACATCTTTGCTAGAAATTTCAAATTTTAAGTATGTGAATTTAGTTAATTAGCTGTGCTGA TACATCTTTGCTAGAAATTTCAAATTTTAAGTATGTGAATTTAGTTAATTAGCTGTGCTGA *****

PR33a	TCAATTCAAAAACATTACTTTCCTAAATTTTAGACTATGAAGGTCATAAATTCAACAAAT
PCGEM1	TCAATTCAAAAACATTACTTTCCTAAATTTTAGACTATGAAGGTCATAAATTCAACAAAT
PR33b	TCAATTCAAAAACATTACTTTCCTAAATTTTAGACTATGAAGGTCATAAATTCAACAAAT

PR33a	ATATCTACACATACAATTATAGATTGTTTTTCATTATAATGTCTTCATCTTAACAGAATT
PCGEM1	ATATCTACACATACAATTATAGATTGTTTTTCATTATAATGTCTTCATCTTAACAGAATT
PR33b	ATATCTACACATACAATTATAGATTGTTTTTCATTATAATGTCTTCATCTTAACAGAATT

PR33a	GTCTTTGTGATTGTTTTTAGAAAACCTGAGAGTTTTAATTCATAATTACTTGATCAAAAAA
PCGEM1	GTCTTTGTGATTGTTTTTAGAAAACCTGAGAGTTTTAATTCATAATTACTTGATCAAAAAA
PR33b	GTCTTTGTGATTGTTTTTAGAAAACCTGAGAGTTTTAATTCATAATTACTTGATCAAAAAA

PR33a	TTGTGGGAACAATCCAGCATTAATTGTATGTGATTGTTTTTATGTACATAAGGAGTCTTA
PCGEM1	TTGTGGGAACAATCCAGCATTAATTGTATGTGATTGTTTTTATGTACATAAGGAGTCTTA
PR33b	TTGTGGGAACAATCCAGCATTAATTGTATGTGATTGTTTTTATGTACATAAGGAGTCTTA

PR33a	AGCTTGGTGCCTTGAAGTCTTTTGTACTTAGTCCCATGTTTAAATTACTACTTTATATC
PCGEM1	AGCTTGGTGCCTTGAAGTCTTTTGTACTTAGTCCCATGTTTAAATTACTACTTTATATC
PR33b	AGCTTGGTGCCTTGAAGTCTTTTGTACTTAGTCCCATGTTTAAATTACTACTTTATATC

PR33a	TAAAGCATTTATGTTTTTCAATTCAATTACATGATGCTAATTATGGCAATTATAACAAA
PCGEM1	TAAAGCATTTATGTTTTTCAATTCAATTACATGATGCTAATTATGGCAATTATAACAAA
PR33b	TAAAGCATTTATGTTTTTCAATTCAATTACATGATGCTAATTATGGCAATTATAACAAA

PR33a	TATTAAAGATTTTCGAAATAGAATATGTGAATTGTTACATACATAGAAATGAAAAGTTCA
PCGEM1	TATTAAAGATTTTCGAAATAGAAAAAAAAAAAAAAAA-----
PR33b	TATTAAAGATTTTCGAAATAGAATATGTGAATTGTTACATACATAGAAATGAAAAGTTCA
	***** * **
PR33a	TTTCGTAAAGCAAGATGCTGGGTGAAAGAGTGCTTTTGATTGAAAGATCACTAGATTAGT
PCGEM1	-----
PR33b	TTTCGTAAAGCAAGATGCTGGGTGAAAGAGTGCTTTTGATTGAAAGATCACTAGATTAGT

PR33a	AGAGGGCAAGACTTCTAGTCCCTAATCTACCCTTAATAGCCATGTGGTCACGTGTAAGTC
PCGEM1	-----
PR33b	AGAGGGCAAGACTTCTAGTCCCTAATCTACCCTTAATAGCCATGTGGTCACGTGTAAGTC

PR33a	AGTGAACCCATCTCATTCTCCTCATACTTTTTTCATCTCTAAAATGAGGGTATAATTTAA
PCGEM1	-----
PR33b	AGTGAACCCATCTCATTCTCCTCATACTTTTTTCATCTCTAAAATGAGGGTATAATTTAA

PR33a	GCTCTTCATTTTTTTTTTTTTTTTGGAGATAGAGTTTTGCTCTTGTCACCCAGGTTGGAGTG
PCGEM1	-----
PR33b	GCTCTTCATTTTTTTTTTTTTTTTGGAGATAGAGTTTTGCTCTTGTCACCCAGGTTGGAGTG

PR33a	CAATGGCACGATCTCAGCTCACTGCAACCCCTGCTTCCTCGGTTCAAGTGATTCTCCTG

PCGEM1	-----
PR33b	CAATGGCACGATCTCAGCTCACTGCAACCCTCTGCTTCCTCGGTTCAAGTGATTCTCCTG
PR33a	CTTCAGCCTCCCAAGTAGCCGGGATTACAGGTGCCCCGCCACCACATCTGGCTAATTTTTTT
PCGEM1	-----
PR33b	CTTCAGCCTCCCAAGTAGCCGGGATTACAGGTGCCCCGCCACCACATCTGGCTAATTTTTTT
PR33a	GTATTTTCACCATGTTGGCCAGGCTGGTCTCGAACCCCTACCTCAGGTGATCCCTCGCCT
PCGEM1	-----
PR33b	GTATTTTCACCATGTTGGCCAGGCTGGTCTCGAACCCCTACCTCAGGTGATCCCTCGCCT
PR33a	CGGCCTCTCAAAGTGCTGGGATTACAGGTGTGAGCCACCACGCCCAGCCCAATATCAGTT
PCGEM1	-----
PR33b	CGGCCTCTCAAAGTGCTGGGATTACAGGTGTGAGCCACCACGCCCAGCCCAATATCAGTT
PR33a	TTTCTTTTTTAAACACAAGGCTAAACACAATCAAAATACTAGCTAGGGGAGAAAAAAAAAAT
PCGEM1	-----
PR33b	TTTCTTTTTTAAACACAAGGCTAAACACAATCAAAATACTAGCTAGGGGAGAAAAAAAAAAT
PR33a	AAGGCACTGTTTATGTGTAACAGGCTCTTGTGCAATCACTGGGCAGACAATAAACAGTA
PCGEM1	-----
PR33b	AAGGCACTGTTTATGTGTAACAGGCTCTTGTGCAATCACTGGGCAGACAATAAACAGTA
PR33a	AGAATCAATCCTTTTCATATATCCTTCTTGCAGAATACATAAAATCCCACAAATGGCTAT
PCGEM1	-----
PR33b	AGAATCAATCCTTTTCATATATCCTTCTTGCAGAATACATAAAATCCCACAAATGGCTAT
PR33a	CTTCCTTTTTTATGATATTTGGAGAATTGTAGCTAAGTGACAGATATTTTGCTTGGGTGTA
PCGEM1	-----
PR33b	CTTCCTTTTTTATGATATTTGGAGAATTGTAGCTAAGTGACAGATATTTTGCTTGGGTGTA
PR33a	TAGACCACAAAGGACTGTGTTTGATGATGGTTGCATAAAATTATACCTTAGTTTTTACT
PCGEM1	-----
PR33b	TAGACCACAAAGGACTGTGTTTGATGATGGTTGCATAAAATTATACCTTAGTTTTTACT
PR33a	TTGTATGTTACATGTTAGATTTAGAGTATGAAAAATTAGTAGGGAGGATTATTAACAAAGA
PCGEM1	-----
PR33b	TTGTATGTTACATGTTAGATTTAGAGTATGAAAAATTAGTAGGGAGGATTATTAACAAAGA
PR33a	ACAGGGCAAGAGGAGTAGAATTAAACCTCTTCTAATACCTGTGCACAAGTAGGCTTTTCA
PCGEM1	-----
PR33b	ACAGGGCAAGAGGAGTAGAATTAAACCTCTTCTAATACCTGTGCACAAGTAGGCTTTTCA
PR33a	GAAACTCTACAACCCTACATAAACTGGATAGTTAGAAAAGCACACTCCCAAGGAAGGCGG
PCGEM1	-----
PR33b	GAAACTCTACAACCCTACATAAACTGGATAGTTAGAAAAGCACACTCCCAAGGAAGGCGG

PR33a	TTATGTTTTGCAGTTTGAATCAGAAGAATAGAGCTATAGCAATCTTCATTCTATAGTAAC
PCGEM1	-----
PR33b	TTATGTTTTGCAGTTTGAATCAGAAGAATAGAGCTATAGCAATCTTCATTCTATAGTAAC
PR33a	ATTAAAGAGCCTGGTTTATATTATAGCAGTCATTAAGATTAAAAATTTACATCTTGCCG
PCGEM1	-----
PR33b	ATTAAAGAGCCTGGTTTATATTATAGCAGTCATTAAGATTAAAAATTTACATCTTGCCG
PR33a	TTCTTCTTACTCACAGATTTTCGAGAGGTAATGTAATGATCCACGAGGTGAGAATCACTG
PCGEM1	-----
PR33b	TTCTTCTTACTCACAGATTTTCGAGAGGTAATGTAATGATCCACGAGGTGAGAATCACTG
PR33a	CCTTTTATAATGCGATTAAATTGCATGAACAAAGTTTCCAACAAATAACAGTAATAAAAA
PCGEM1	-----
PR33b	CCTTTTATAATGCGATTAAATTGCATGAACAAAGTTTCCAACAAATAACAGTAATAAAAA
PR33a	GAAACATGTATTAGCACTTAATAAGCCAGGGGCTGGACGACGTGTGTTACATGCTTTCAA
PCGEM1	-----
PR33b	GAAACATGTATTAGCACTTAATAAGCCAGGGGCTGGACGACGTGTGTTACATGCTTTCAA
PR33a	TCCATGAACTGGTAAACTGGTACTAGTATCTCTATTGGACATGTGAGGAAACCAAATGGA
PCGEM1	-----
PR33b	TCCATGAACTGGTAAACTGGTACTAGTATCTCTATTGGACATGTGAGGAAACCAAATGGA
PR33a	GTTGATAAACAGTAGAGTTAAAAATTACTCTTCATATATTATATTGCCTCAATCTCACAG
PCGEM1	-----
PR33b	GTTGATAAACAGTAGAGTTAAAAATTACTCTTCATATATTATATTGCCTCAATCTCACAG
PR33a	ACATCTCTGCTACCAAAGCTATCATATCTAGATATGCGGCATAAGGATGACCTTGGGGC
PCGEM1	-----
PR33b	ACATCTCTGCTACCAAAGCTATCATATCTAGATATGCGGCATAAGGATGACCTTGGGGC
PR33a	ACACTAGAATTCTTTGAGAGAATTCTGGCAGAGAAAACAAATATTTATTCTACAATAAA
PCGEM1	-----
PR33b	ACACTAGAATTCTTTGAGAGAATTCTGGCAGAGAAAACAAATATTTATTCTACAATAAA
PR33a	ACCCAGCATTTTACAGGTTTTATTTTAACTATGAAGTATTGTTATCTGTATCTTTCATA
PCGEM1	-----
PR33b	ACCCAGCATTTTACAGGTTTTATTTTAACTATGAAGTATTGTTATCTGTATCTTTCATA
PR33a	TAAGTGTGCCCCGAATTTATTTCTTCTGGTGGGTTCTTGGTCTCGCTGACTCCAAGAATG
PCGEM1	-----
PR33b	TAAGTGTGCCCCGAATTTATTTCTTCTGGTGGGTTCTTGGTCTCGCTGACTCCAAGAATG

PR33a	AAACCGCAGACCCTTGAGGTGAGTGTACAGTTCTTAAAGATGGTGTGTTTCAGAGTTTGT
PCGEM1	-----
PR33b	AAACCGCAGACCCTTGAGGTGAGTGTACAGTTCTTAAAGATGGTGTGTTTCAGAGTTTGT
PR33a	TCCTTCAGATGTTTCAGATGTGTCCGGAGTTTCTCCCTTATGGTGAGTTCGTGGTCTCGCT
PCGEM1	-----
PR33b	TCCTTCAGATGTTTCAGATGTGTCCGGAGTTTCTCCCTTATGGTGAGTTCGTGGTCTCGCT
PR33a	GACTTCAACAATGAAGCCGCAGACCTTTGCAGTGAGTGTGTGACAGTTCTTAAAGGCAGT
PCGEM1	-----
PR33b	GACTTCAACAATGAAGCCGCAGACCTTTGCAGTGAGTGTGTGACAGTTCTTAAAGGCAGT
PR33a	GCGTCCAGAGTTGTTTGTTCCTCCCGGTAGGTTTCGTGGTCTCGCTGATGTCAGGAATGAA
PCGEM1	-----
PR33b	GCGTCCAGAGTTGTTTGTTCCTCCCGGTAGGTTTCGTGGTCTCGCTGATGTCAGGAATGAA
PR33a	GCTGCAGACCCTCGCGGTAAGTGTTACAGCTCATAAAGGTAGTGCAAACCCAAACAGTGA
PCGEM1	-----
PR33b	GCTGCAGACCCTCGCGGTAAGTGTTACAGCTCATAAAGGTAGTGCAAACCCAAACAGTGA
PR33a	GCAGTAGCAAGATTTATTATGAAGAGCAAAAGAAACAAAGCTTCCCCACCATAGAAACGGA
PCGEM1	-----
PR33b	GCAGTAGCAAGATTTATTATGAAGAGCAAAAGAAACAAAGCTTCCCCACCATAGAAACGGA
PR33a	CCAGAATTGGTTGCTGCTGCTGTGGTAGCCAGCTTTTATTCCCTTATTTGGCCACACCCA
PCGEM1	-----
PR33b	CCAGAATTGGTTGCTGCTGCTGTGGTAGCCAGCTTTTATTCCCTTATTTGGCCACACCCA
PR33a	CATCCTGCTGATTGGGCCATTTTACAGAATGCTGATTGGTCCATTTTATAGCGTGCTGAT
PCGEM1	-----
PR33b	CATCCTGCTGATTGGGCCATTTTACAGAATGCTGATTGGTCCATTTTATAGCGTGCTGAT
PR33a	TGGTGCGTTTTTTACAGAGTGCTGATTGGTGCATTTACAATCCTTTAGCTAGACACAGAGT
PCGEM1	-----
PR33b	TGGTGCGTTTTTTACAGAGTGCTGATTGGTGCATTTACAATCCTTTAGCTAGACACAGAGT
PR33a	GCTGATTGGTGCCTTTTATAATCCTTTAGCTAGACACAAAAGTTCTACAAGTCCCCACCCA
PCGEM1	-----
PR33b	GCTGATTGGTGCCTTTTATAATCCTTTAGCTAGACACAAAAGTTCTACAAGTCCCCACCCA
PR33a	ACCCAGAAGCTCCGCTGGCTTCACCTCTCGTAAGGAAATTGAGGTTCAAACAAGTTTCAA
PCGEM1	-----
PR33b	ACCCAGAAGCTCCGCTGGCTTCACCTCTCGTAAGGAAATTGAGGTTCAAACAAGTTTCAA
PR33a	AGTGCTAAAACCTACAGTTTCTCATTCTCTGCAACTGGATTTCCTCATGTGTTTGAATC
PCGEM1	-----

PR33b	AGTGCTAAAACTACAGTTTCTCATTCTCTGCAACTGGATTTCCACTCATGTGTTTGAATC
PR33a	CCAGGCTCTAAGACTTAACTTGCCATTCTGTGACTTTATGTTCTGCAATTTACACAAAAG
PCGEM1	-----
PR33b	CCAGGCTCTAAGACTTAACTTGCCATTCTGTGACTTTATGTTCTGCAATTTACACAAAAG
PR33a	CTACTATCTGTACATCTCTGGTGTTAACTTCAGACTAAACTTCTTTTTGATTACACAATG
PCGEM1	-----
PR33b	CTACTATCTGTACATCTCTGGTGTTAACTTCAGACTAAACTTCTTTTTGATTACACAATG
PR33a	ACCACACACTTTTTGGTTGAGGTTTTGCTATCGGTTTATTGTACTGGTTAATAGAGAGCT
PCGEM1	-----
PR33b	ACCACACACTTTTTGGTTGAGGTTTTGCTATCGGTTTATTGTACTGGTTAATAGAGAGCT
PR33a	TCTTCCAGAAATTTGAGTAGATGGAAGAGGAAGTAGCACATTCTTAAAAATGTACCATGC
PCGEM1	-----
PR33b	TCTTCCAGAAATTTGAGTAGATGGAAGAGGAAGTAGCACATTCTTAAAAATGTACCATGC
PR33a	CTTTCAAGTCACAAGCATCCCTATCACATGGCTGTCAAGGGTGGCTCAGAATAGGTAGAG
PCGEM1	-----
PR33b	CTTTCAAGTCACAAGCATCCCTATCACATGGCTGTCAAGGGTGGCTCAGAATAGGTAGAG
PR33a	TTAAGAATTTAAAGTAAATTGGTGTAAGCGATGAAAGCTTCATCTAAAAGCTTATATTAC
PCGEM1	-----
PR33b	TTAAGAATTTAAAGTAAATTGGTGTAAGCGATGAAAGCTTCATCTAAAAGCTTATATTAC
PR33a	ATCAACTGAAATGTAAAATAATTGGAACATTTTCCAGGCATCCCTGTTATTTATTTGTCT
PCGEM1	-----
PR33b	ATCAACTGAAATGTAAAATAATTGGAACATTTTCCAGGCATCCCTGTTATTTATTTGTCT
PR33a	CTCTTTCCTTGCTTGCCTACTTCAAAAGTCATATGGCATGGTGACTAGAACTGTCCTGCC
PCGEM1	-----
PR33b	CTCTTTCCTTGCTTGCCTACTTCAAAAGTCATATGGCATGGTGACTAGAACTGTCCTGCC
PR33a	AAAGAGTTTGTCAATATAAGATTCCTTTCTTTGTAAACATTCTACCTGGGGCTTCATTT
PCGEM1	-----
PR33b	AAAGAGTTTGTCAATATAAGATTCCTTTCTTTGTAAACATTCTACCTGGGGCTTCATTT
PR33a	ATAATCAAAAGGAGTACTGTAACCTGTCAAAAAAAGCTACCTGTGACAATATATTATGT
PCGEM1	-----
PR33b	ATAATCAAAAGGAGTACTGTAACCTGTCAAAAAAAGCTACCTGTGACAATATATTATGT
PR33a	GATGGTTACCTGCAGTAAGGTGGTGGCAATAAATAAATAAATAATCACAGAATGAAACCG
PCGEM1	-----
PR33b	GATGGTTACCTGCAGTAAGGTGGTGGCAATAAATAAATAAATAATCACAGAATGAAACCG

PR33a	AGCAGAACTGTCAGAGAAATGGTCAGAATTCACACTCTGAAGAACACGGCTATACAGTAA
PCGEM1	-----
PR33b	AGCAGAACTGTCAGAGAAATGGTCAGAATTCACACTCTGAAGAACACGGCTATACAGTAA
PR33a	TAATCATAATAAATAGCCACTCAATCCAAAACATCACTGGGCGACTTGTCACATATATAA
PCGEM1	-----
PR33b	TAATCATAATAAATAGCCACTCAATCCAAAACATCACTGGGCGACTTGTCACATATATAA
PR33a	TCAGTGGAGATGTGATTGAAGCACAAAGGCTTAAGTGAATGTCTAGAGAGCTAATTGATTC
PCGEM1	-----
PR33b	TCAGTGGAGATGTGATTGAAGCACAAAGGCTTAAGTGAATGTCTAGAGAGCTAATTGATTC
PR33a	ATTTTTATGGAAATTTTACTTATTTTAAATGTCATCCCTGACCATCTTGAACTTTACTT
PCGEM1	-----
PR33b	ATTTTTATGGAAATTTTACTTATTTTAAATGTCATCCCTGACCATCTTGAACTTTACTT
PR33a	GAAGATTTATTTTTTTTTTTTAAATCACTGTTTATTAGATTTAGGTATTCTGGTCTTTGTT
PCGEM1	-----
PR33b	GAAGATTTATTTTTTTTTTTTAAATCACTGTTTATTAGATTTAGGTATTCTGGTCTTTGTT
PR33a	TTTCTTTTTTATCTATGTATGATTTTTATTTTTTTATGCAGTGTCTTAAGCTTCATCAA
PCGEM1	-----
PR33b	TTTCTTTTTTATCTATGTATGATTTTTATTTTTTTATGCAGTGTCTTAAGCTTCATCAA
PR33a	TGAGAAGAAATGTATTAAAATCCATTTATTCTTACCCTAAAAAAAAAAAAAAAAAAAAA
PCGEM1	-----
PR33b	TGAGAAGAAATGTATTAAAATCCATTTATTCTTACCCTAAAAAAAAAAAAAAAAAAAAA

SHORT REPORTS

Elevated expression of *PCGEM1*, a prostate-specific gene with cell growth-promoting function, is associated with high-risk prostate cancer patients

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PCGEM1 is a novel, highly prostate tissue-specific, androgen-regulated gene. Here, we demonstrate that *PCGEM1* expression is significantly higher in prostate cancer (CaP) cells of African-American men than in Caucasian-American men ($P=0.0002$). Further, increased *PCGEM1* expression associates with normal prostate epithelial cells of CaP patients with a family history of CaP ($P=0.0400$). *PCGEM1* overexpression in LNCaP and in NIH3T3 cells promotes cell proliferation and a dramatic increase in colony formation, suggesting a biological role of *PCGEM1* in cell growth regulation. Taken together, the cell proliferation/colony formation-promoting functions of *PCGEM1* and the association of its increased expression with high-risk CaP patients suggest the potential roles of *PCGEM1* in CaP onset/progression, especially in these high-risk groups.

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Introduction

Prostate cancer (CaP) is a complex, multifactorial disease with heterogeneous tumor types, making prediction of the clinical course for individual CaP patients a difficult challenge (Small, 1998; Isaacs *et al.*, 2002). Traditional prognostic markers, such as Gleason grade, clinical stage, and pretreatment prostate-specific antigen (PSA) levels have only limited prognostic value for an individual patient (Small, 1998). The established risk factors for CaP are patient age, ethnic origin, and CaP family history (Isaacs *et al.*, 2002). The molecular mechanisms of CaP susceptibility are being currently defined (Isaacs *et al.*, 2002; Xu *et al.*, 2002).

Among several approaches to define CaP-specific genetic alterations, comparison of global gene expression profiles in cancer cells and corresponding normal cells is emerging as a successful strategy, revealing consistent overexpression of *HEPSIN* (Dhanasekaran *et al.*, 2001; Magee *et al.*, 2001; Welsh *et al.*, 2001; Luo *et al.*, 2002) and *AMACR* (Rubin *et al.*, 2002) in CaP, and *EZH2* (Varambally *et al.*, 2002) in metastatic CaP. Recently, the expression pattern of a group of five genes was reported to correlate with CaP progression (Singh *et al.*, 2002). Gene expression alterations in tumor cells may also predispose to cancer (Yan *et al.*, 2002). CaP-specific global gene expression analyses in our laboratory have defined *PCGEM1* as a highly prostate-specific, androgen-regulated gene with cancer-associated overexpression (Srikantan *et al.*, 2000).

PCGEM1 appears to be a noncoding functional RNA gene (Srikantan *et al.*, 2000). *PCGEM1* is similar to *DD3* (Bussemakers *et al.*, 1999) in that these genes are highly prostate-specific, nonprotein-coding genes. However, no sequence homology exists between *PCGEM1* and *DD3*. Recent reviews of the literature (Szymanski and Barciszewski, 2002) and a database of noncoding RNAs demonstrate (<http://biobases.ibch.poznan.pl/ncRNA/>) that an increasing number of noncoding RNA genes are being discovered that may have biological functions in diverse cellular processes. *H19*, *His-1*, and *Bic* represent examples of noncoding RNAs implicated in tumorigenesis (Szymanski and Barciszewski, 2002). In this regard, *PCGEM1* and *DD3* may represent a new class of prostate-specific genes. Therefore, our laboratory is pursuing an in-depth evaluation of the biological functions of *PCGEM1* and features of its tumor-associated expression.

In order to gain an insight into the cell biologic function of *PCGEM1*, NIH3T3 and LNCaP cells overexpressing *PCGEM1* were generated. Cells were transfected with *PCGEM1* cDNA cloned into a eucaryotic expression vector (pEAK8). Puromycin-resistant transfectants were selected and pooled cell lines stably overexpressing *PCGEM1* have been established. The strong expression of *PCGEM1* RNA in both

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the LNCaP and the NIH3T3 transfectants was detectable by Northern blot analysis (Figures 1a and 2a Inset). Control cells were transfected with the vector without the *PCGEM1* insert.

The effects of *PCGEM1* overexpression on cell growth/proliferation and cell cycle were evaluated. NIH 3T3 and LNCaP transfectants overexpressing *PCGEM1* were plated at low density in 96-well plates. Cell growth was followed by either counting the cell number or by a colorimetric assay. Both LNCaP cells (Figure 1a) and NIH3T3 cells (Figure 2a) overexpressing *PCGEM1* exhibited a highly significant increase in proliferation compared to the vector-control-transfected cells ($P < 0.001$, calculated by *t*-test). Further, in colony-forming assays with both LNCaP cells (Figure 1b) and NIH3T3 cells (Figure 2b), overexpression of *PCGEM1* led to a dramatic increase in colony formation compared to the vector control.

The effect of *PCGEM1* overexpression on cell cycle was analysed using a panel of phosphorylation-specific antibodies raised against key cell-cycle-related proteins in Western blot experiments (CDC2, CDC25, p53, Rb Ser780, Rb Ser795, Rb Ser807/811, cyclin D1, and Chk1). In both LNCaP (Figure 1c) and NIH 3T3 (Figure 2c) cells overexpressing *PCGEM1*, a significant increase in Rb phosphorylation (Ser807/811) was detected, indicating that *PCGEM1* overexpression may affect cell proliferation through Rb phosphorylation. Rb Ser807/811 is often phosphorylated in uveal melanoma, the most common malignancy of the eye, and Rb is functionally inactivated by this phosphorylation (Brantley and Harbour, 2000). Rb Ser807/811 phosphorylation has been shown to disrupt Rb binding to the protooncogene *c-abl* (Knudsen and Wang, 1996). Further experiments are needed to reveal the signal transduction pathway involving *PCGEM1* and Rb Ser807/811.

The cell proliferation/colony formation-promoting effects of *PCGEM1* overexpression in the LNCaP prostate cell line suggest that it may have a functional role in cell growth regulation in human CaP cells. Sequence analysis of mouse and rat genome sequences revealed a 131 bp region of strong homology to human *PCGEM1* (83% to mouse, 85% to rat), which weakens (about 60%) with several gaps in the surrounding regions. The homology exists only at the DNA/RNA level; no possible peptides appear to be conserved between the different species. The *PCGEM1* genomic sequence homology is between corresponding regions of human chromosome 2, mouse chromosome 1, and rat chromosome 9. RNA secondary structure prediction analysis (Mfold) indicated that *PCGEM1* RNA has a lower free energy state than coding genes of the same size, or randomized versions of the *PCGEM1* sequence. Within the 1.6 kb *PCGEM1* cDNA, the first half (1–800 bp) had much lower predicted free energy (–221) than the second half (–163), indicating that the presence of a low-energy, stable secondary RNA structure is more likely in the first 800 bp region of *PCGEM1*. Mutational analysis of *PCGEM1* as well as efforts to identify intracellular molecules binding to *PCGEM1* RNA are in progress in our laboratory.

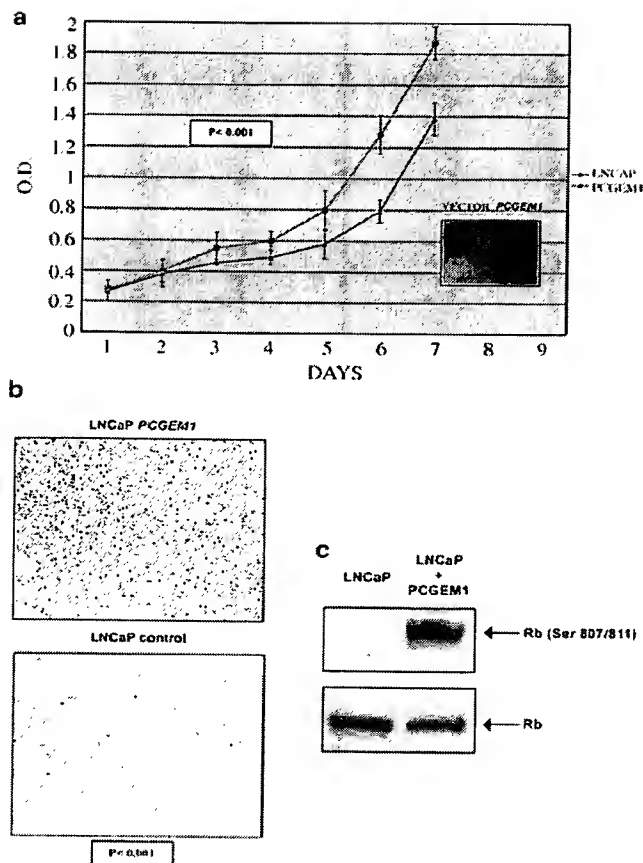


Figure 1 Increased cell proliferation and colony formation by LNCaP cells overexpressing *PCGEM1*. (a) Cell proliferation (plotted as optical density, measured by CellTiter Proliferation Kit, Promega) and (b) colony formation of LNCaP cells overexpressing *PCGEM1*. Inset: Detection of *PCGEM1* RNA by Northern blot in LNCaP *PCGEM1* transfectants. A total of 1000 cells/well were plated in 96-well plates, and cell proliferation was measured each day for 7 days in triplicate for each cell line. *P*-values for data-point average pairs were determined by the *t*-test. The $P < 0.0001$ value (shown in the figure) is reached by Day 6 and Day 7. For the colony formation assay, 3000 cells were plated in 100 mm Petri dishes or T-75 cell culture flasks for each cell line. After 2 weeks, the developing individual colonies were stained with crystal violet. The number of colonies formed after 2 weeks by LNCaP parent cells (250–280) and LNCaP cells overexpressing *PCGEM1* (3000–3600) were counted in three independent experiments, and the average values were analysed by the *t*-test. The full-length *PCGEM1* cDNA was obtained in a eucaryotic expression vector (pEAK8, Edge BioSystems) by screening a prostate cDNA library (Edge BioSystems). A sequence-verified *PCGEM1* cDNA gene in the pEAK8 expression vector was transfected into both LNCaP and NIH3T3 cells using lipofectamine (Invitrogen, Carlsbad, CA, USA). Transfectants were selected by 0.2 mg/l (LNCaP) or 5 mg/l (NIH3T3) puromycin (Edge BioSystems). (c) Detection of Rb phosphorylation in LNCaP cells with and without *PCGEM1* expression. Protein lysates from exponentially growing cells were quantitated using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and 30 μ g aliquots were subjected to Western blotting. Antibodies raised against phosphorylated forms of cell cycle-related proteins CDC2, CDC25, p53, Rb Ser780, Rb Ser795 and Rb Ser807/811, Chk1, as well as the Rb control antibody, were obtained from Cell Signaling Technology (Beverly, MA, USA).

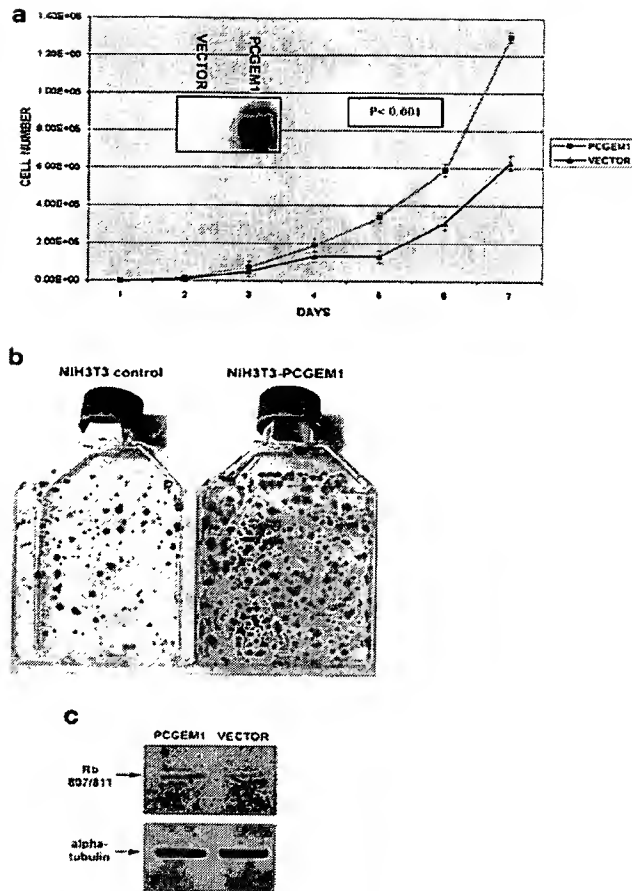


Figure 2 Cell proliferation and colony formation of NIH3T3 cells increases with *PCGEM1* overexpression. (a) Cell proliferation (plotted as daily cell number counts) and (b) colony formation of NIH3T3 cells overexpressing *PCGEM1*. Experiments were performed as described in Figure 1. Inset: Detection of *PCGEM1* RNA by Northern blot in NIH3T3 *PCGEM1* transfectants. (c) Detection of Rb phosphorylation in NIH3T3 cells with and without *PCGEM1* expression using the same procedure as described in Figure 1c. Alpha-tubulin antibody served as an internal control (Santa Cruz Biotechnology, Santa Cruz, CA, USA)

Although the number of noncoding RNA genes identified has sharply increased in recent years (<http://biobases.ibch.poznan.pl/ncRNA/>), functional data are available only on a small subset of these genes (Szymanski and Barciszewski, 2002). H19, one of the most well-characterized noncoding RNAs, exhibited tumor suppressor activity when overexpressed in embryonal carcinoma cells (Hao *et al.*, 1993). Recent results, however, indicated that in breast epithelial cells, H19 overexpression promotes tumor progression without affecting cell proliferation (Lottin *et al.*, 2002). His-1 (Askew *et al.*, 1994) and Bic (Tam *et al.*, 1997) are noncoding RNA genes whose transcription is activated by retroviral insertion, and they are implicated in the pathogenesis of hematological malignancies. Avian bic

cooperates with *c-myc* in oncogenesis, and in enhancing the growth of chicken embryo fibroblasts (Tam *et al.*, 2002). No functional data are reported on the prostate-specific noncoding gene DD3, which has a highly CaP-specific expression (Bussemakers *et al.*, 1999).

In an attempt to assess potential clinical utility of *PCGEM1*, its expression levels in both cancer and normal prostate epithelial cells derived from radical prostatectomy specimens of CaP patients were analysed. Using OCT-embedded frozen sections of prostate tissues, prostate epithelial cells with normal and cancer phenotypes, as defined by hematoxylin-eosin (H&E) staining, were laser capture microdissected (LCM). A total of 180 RNA specimens representing microdissected paired normal and tumor cells of 90 CaP patients were quantified and used for expression analyses.

As a quality control, each LCM RNA sample was assayed by RT-PCR for the expression of NKX 3.1, a prostate epithelial cell marker (Xu *et al.*, 2000). In a subset of 40 patients, the expression of *DD3*, a sensitive and specific marker of prostate tumor cells (DeKok *et al.*, 2002), was also determined. Over 80% of the patients exhibited higher *DD3* expression in their prostate tumor cells than in the normal prostate epithelium (data not shown). This bank of paired normal and tumor cell-derived RNAs were screened by real-time multiplex quantitative RT-PCR (TaqMan) for the expression of *PCGEM1*. The expression data were normalized to *GAPDH* expression levels, which was measured in parallel in the same tubes (multiplex PCR).

The association of *PCGEM1* expression data with 35 different clinicopathological parameters linked to the CaP patients were analysed (full list in legends to Table 1). A summary of *PCGEM1* association with selected clinicopathological features is presented in Table 1. The statistical analyses revealed a striking association between *PCGEM1* expression levels and the ethnic origin of CaP patients. Tumor cells of African-American CaP patients ($n=22$) harbored significantly higher *PCGEM1* expression ($P=0.0002$) compared to those of Caucasian-American CaP patients ($n=66$) (Figure 3a). In contrast, *PCGEM1* expression in normal prostate epithelial cells of these two patient groups was not significantly different ($P=0.6001$) (Figure 3b). In the African-American patient population, *PCGEM1* expression was increased in their prostate tumor cells compared to matched normal prostate epithelial cells in 68.2% of the cases (15/22). On the other hand, 41% (27/66) of Caucasian-American CaP patients showed tumor cell-associated *PCGEM1* overexpression in comparison to matched normal epithelial cells. To our knowledge, this is the first observation of a prostate-specific gene with a cell growth-promoting function that shows elevated expression in African-American CaP patients, the population with the highest CaP incidence in the world. At this point, however, we cannot conclude that *PCGEM1* overexpression is cancer specific, because *PCGEM1* expression has not been evaluated in other pathological conditions of the prostate (BPH, prostatitis).

Table 1 Relationship of *PCGEM1* expression in prostate epithelial cells to clinicopathological features of CaP patients undergoing radical prostatectomy

Clinicopathological features (n)	Tumor cells		Normal cells		P-values	
	<i>PCGEM1</i> expression		<i>PCGEM1</i> expression		Tumor	Normal
	0-3 'low' Patient number (n = 90)	4-5 'high' Patient number (n = 90)	0-3 'low' Patient number (n = 90)	4-5 'high' Patient number (n = 90)		
Cell differentiation	58	31	61	28	0.1138	0.6158
Well (46)	32	14	31	15		
Moderate (28)	14	14	18	10		
Poor (15)	12	3	12	3		
Race	59	31	62	28	0.0002	0.6001
African-American (22)	7	15	14	8		
Caucasian-American (66)	51	15	46	20		
Age (years)	59	31	62	28	0.4100	0.5260
40-54 (29)	12	8	16	4		
55-59 (23)	18	5	16	7		
60-64 (27)	18	9	16	11		
65-75 (20)	11	9	14	6		
Prebiopsy PSA	58	31	61	28	0.2031	0.6705
0-4 (11)	9	2	7	4		
4.1-7 (42)	25	12	26	11		
7.1-10 (18)	13	5	14	4		
10.1+ (23)	11	12	14	9		
Family history of CaP	55	28	56	27	0.7984	0.0400
Yes (24)	15	9	12	12		
No (59)	40	19	44	15		
T stage	57	30	60	27	0.6016	0.6476
T2 (33)	24	9	22	11		
T3a, b (39)	24	15	26	13		
T3c (15)	9	6	12	3		
Margins	58	31	61	28	0.8174	0.1468
Neg (59)	39	20	37	22		
Pos (30)	19	11	24	6		
Seminal vesicle	58	31	61	28	0.3745	0.3723
Neg (74)	50	24	49	25		
Pos (15)	8	7	12	3		

RNA isolated (MicroRNA kit, Stratagene, La Jolla, CA, USA) from LCM samples of tumor and normal epithelial cells of radical prostatectomy patients were quantitated (RiboGreen dye, Molecular Probes, Eugene, OR and VersaFluor fluorimeter, BioRad, Hercules, CA, USA) and assayed by real-time quantitative RT-PCR (TaqMan). In all, 1 ng LCM RNA was used to produce cDNA, sufficient for 10 PCR reactions. Real-time quantitative PCR analysis was performed using TaqMan detection chemistry on the ABI Prism 7700 Sequence Detection System according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). PCR primers were: forward primer, 5'-TTCAATTAGGCAG CAACCTTT-3'; reverse primer, 5'-CATTGAGCTCTATGAATCTGCTTAA-3'; and Taqman probe, FAM-CCGTAACCTGTGTCTG CAACTTCCTCTAATT-TAMRA. Each PCR was multiplexed using the GAPDH detection control mix (Applied Biosystems, Foster City, CA, USA) as an internal control in the same tube with the *PCGEM1* mix. GAPDH control cycle threshold (C_T) values obtained from the real-time PCR assays were subtracted from the *PCGEM1* C_T values. The resulting values represent *PCGEM1* expression levels normalized to GAPDH (dC_T values). Normalized *PCGEM1* expression levels were categorized as 1 ($dC_T > 10$), 2 ($6 < dC_T < 10$), 3 ($3 < dC_T < 6$), 4 ($1 < dC_T < 3$), 5 ($dC_T < 1$), and 0 (no detectable *PCGEM1* expression). Statistical analysis was performed with the SAS software package (SAS Institute Inc., Cary, NC, USA), comparing *PCGEM1* expression categories 0-3 ('low' and no *PCGEM1* expression) to categories 4-5 ('high' *PCGEM1* expression). The association between *PCGEM1* expression and clinicopathological features was analysed using Fisher's exact test. The number of patients is in brackets. $P < 0.05$ was considered statistically significant. The prostate tissue specimens were obtained under an IRB-approved protocol from patients treated at Walter Reed Army Medical Center (WRAMC) and Uniformed Services University of the Health Sciences (USUHS). The patient database included the following clinicopathological parameters: race, prebiopsy PSA, diagnosis date, surgery date, age at surgery, left Gleason sum, right Gleason sum, worst Gleason sum, prostatitis at diagnosis, PIN, HGPIN, pretreatment testosterone, family history of CaP, T stage, neoadjuvant date, margins, tumor number; PSA recurrence: months after surgery, date, prostatitis pathology; bone metastasis: months after surgery, date; follow-up: months after surgery, latest contact date, capsule, seminal vesicle, nodes, worst grade, bladder neck, multifocal, diagnosis PSA, worst nuclear grade, prostate weight, hormone refractory date, differentiation at LCM microenvironment

Genetic, hormonal, and environmental factors may contribute to a higher risk of CaP in African-American men (Brawley *et al.*, 1998; Powell, 1998; Cussenot and

Valeri, 2001). Polymorphism of genes, for example, CYP3A4 (Paris *et al.*, 1999), androgen receptor (Platz *et al.*, 2000), and vitamin D receptor (Taylor *et al.*,

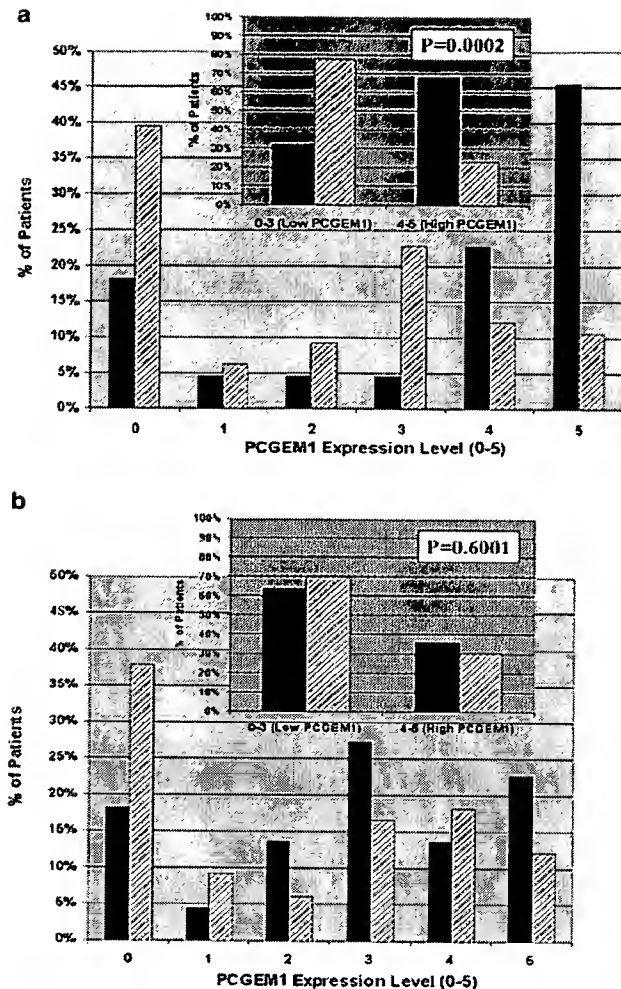


Figure 3 *PCGEM1* expression in microdissected tumor (a) and normal (b) prostate epithelial cells of African-American and Caucasian-American CaP patients. *PCGEM1* expression data were obtained and analysed as described in Table 1. The percentage of African-American (black columns) and Caucasian-American (striped columns) CaP patients expressing *PCGEM1* at different levels (0–5) in their prostate epithelial cells are represented by the columns. The *P*-value (in box) was calculated by Fisher's exact test

1996), may predispose African-American men to higher CaP risk in comparison to Caucasian-Americans. Bcl-2, an antiapoptotic protein (Guo *et al.*, 2000) and caveolin-1, a membrane protein with tumor suppressor activity (Yang *et al.*, 2000), show differential expression in CaP cells of African-American men compared to Caucasian-Americans. *PCGEM1* belongs to this latter category, with several interesting features: it is a prostate-specific gene, it promotes cell proliferation/colony formation, and it shows highly significant prostate tumor cell-specific overexpression in African-Americans versus Caucasian-Americans.

It is worth noting that increased PSA levels in the serum of African-American men compared to that of

Caucasian-Americans have been described (Moul *et al.*, 1995). Although *PCGEM1* is an androgen-regulated gene, we found no significant association between pretreatment PSA levels and *PCGEM1* expression in either tumor ($P=0.2031$) or normal ($P=0.6705$) prostate epithelial cells (Table 1), making it unlikely that a general induction of the androgen pathway would be responsible for the elevated *PCGEM1* RNA levels.

Intriguingly, our analysis of the relative levels of *PCGEM1* in histologically normal cells of CaP patients revealed a significant increase in *PCGEM1* expression in patients with a family history of CaP (12/24), as

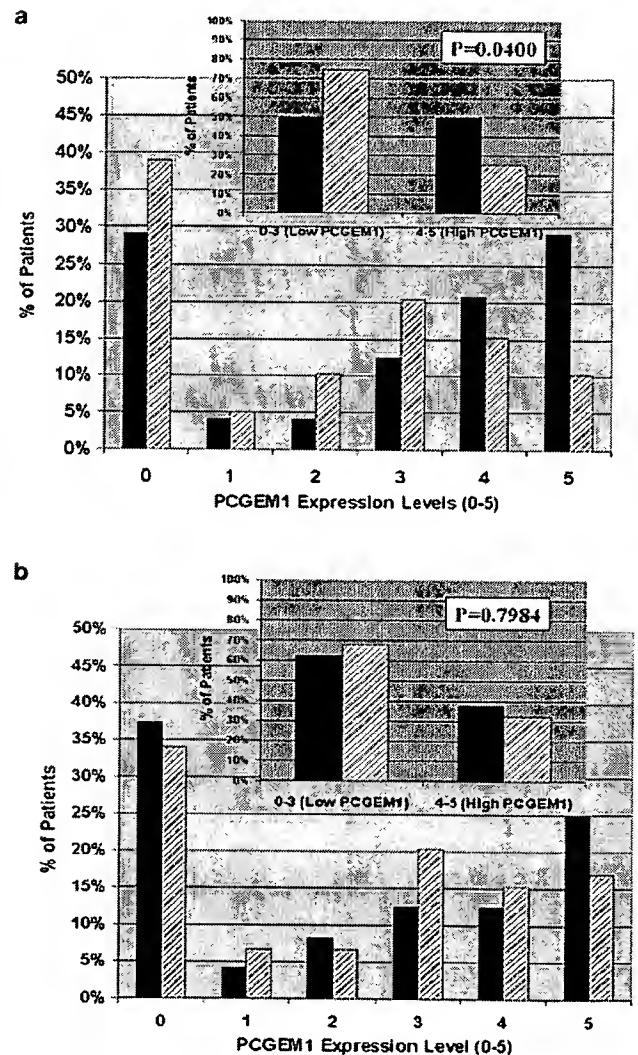


Figure 4 *PCGEM1* expression in microdissected tumor (a) and normal (b) prostate epithelial cells of CaP patients with and without family history of CaP. *PCGEM1* expression data were obtained and analysed as described in Table 1. The percentage of patients with (black columns) and without (striped columns) a family history of CaP expressing *PCGEM1* at different levels (0–5) in their prostate epithelial cells are represented by the columns. The *P*-value (in box) was calculated by Fisher's exact test

opposed to patients with no family history (15/59) ($P=0.0400$) (Figure 4).

A multivariable logistic regression analysis with backward elimination of insignificant variables was performed for the association of *PCGEM1* expression with patient characteristics (race, family history of CaP, margin, prebiopsy PSA, prostate weight, worst Gleason sum, differentiation). The analysis revealed that the African-American race is significantly related to *PCGEM1* expression in tumor cells ($P=0.0007$), and a family history of CaP is significantly related to *PCGEM1* expression in normal prostate epithelial cells ($P=0.0374$). All other variables analysed were insignificant.

Potential CaP biomarkers that are consistently over-expressed in CaP cells, for example, *HEPSIN*, *AMACR*, *DD3*, and *PSMA*, do not show any reported association with ethnicity or family history of CaP (Isaacs et al., 2002). Loss of *GSTP1* expression is also among the most common alterations in CaP cells (Nelson et al., 2001). *PCGEM1* appears to be a distinct prostate-specific gene associated with these high-risk CaP patients. Only a few studies on cancer-associated expression of other non-coding RNA genes have been reported. H19 appears to be lost in certain embryonal tumors (Hao et al., 1993), but overexpressed in a small subset of breast cancers (Lottin et al., 2002). Retroviral insertion events appear to activate the expression of Bic (Tam et al., 1997) and of His-1 (Askew et al., 1994), but cancer-associated

expression of these genes in humans has not been reported. The expanding universe of noncoding RNAs and their functional evaluations may define additional genes with potential functions in the process of tumorigenesis.

Taken together, the cell proliferation/colony formation-promoting function of a novel prostate-specific noncoding gene, *PCGEM1*, and the association of its increased expression level with high-risk CaP patients suggest the potential roles of *PCGEM1* in CaP biology. Further, *PCGEM1* expression characteristics may provide a promising biomarker and potential therapeutic target in the high-risk CaP patients noted herein.

Abbreviations

CaP, prostate cancer; PSA, prostate-specific antigen; FBS, fetal bovine serum; LCM, laser capture microdissection; RT, reverse transcription; H&E, hematoxylin-eosin.


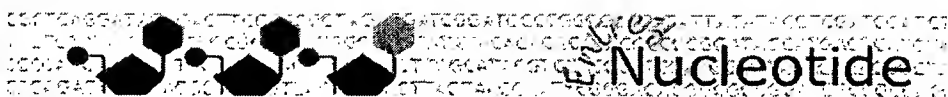
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References

- Askew DS, Li J and Ihle JN. (1994). *Mol. Cell. Biol.*, **14**, 1743–1751.
- Brantley MA and Harbour JW. (2000). *Cancer Res.*, **60**, 4320–4323.
- Brawley OW, Knopf K and Thompson I. (1998). *Semin. Urol. Oncol.*, **16**, 193–201.
- Bussemakers MJ, van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HF, Schalken JA, Debruyne FM, Ru N and Isaacs WB. (1999). *Cancer Res.*, **59**, 5975–5979.
- Cussenot O and Valeri A. (2001). *Eur. J. Intern. Med.*, **12**, 11–16.
- DeKok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeny LA, Aalders TW, Swinkels DW and Schalken JA. (2002). *Cancer Res.*, **62**, 2695–2698.
- Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, Pienta KJ, Rubin MA and Chinnaiyan AM. (2001). *Nature*, **412**, 822–826.
- Guo Y, Sigman DB, Borkowski A and Kyprianou N. (2000). *Prostate*, **42**, 130–136.
- Hao Y, Crenshaw T, Moulton T, Newcomb E and Tycko B. (1993). *Nature*, **365**, 764–767.
- Isaacs W, De Marzo A and Nelson WG. (2002). *Cancer Cell*, **2**, 113–116.
- Knudsen ES and Wang JY. (1996). *J. Biol. Chem.*, **271**, 8313–8320.
- Lottin S, Adrianssens E, Dupressoir T, Berteaux N, Montpeller C, Coll J, Dugimont T and Curgy JJ. (2002). *Carcinogenesis*, **23**, 1885–1895.
- Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, Ewing CM, Platz EA, Ferdinandusse S, Wanders RJ, Trent JM, Isaacs WB and De Marzo AM. (2002). *Cancer Res.*, **62**, 2220–2226.
- Magee JA, Araki T, Patil S, Ehrig T, True L, Humphrey PA, Catalona WJ, Watson MA and Milbrandt J. (2001). *Cancer Res.*, **61**, 5692–5696.
- Moul JW, Sesterhenn IA, Connelly RR, Douglas T, Srivastava S, Mostofi FK and McLeod DG. (1995). *JAMA*, **274**, 1277–1281.
- Nelson WG, De Marzo AM, Dewese TL, Lin X, Brooks JD, Putzi MJ, Nelson CP, Groopman JD and Kensler TW. (2001). *Ann. NY Acad. Sci.*, **952**, 135–144.
- Paris PL, Kupelian PA, Hall JM, Williams TL, Levin H, Klein EA, Casey G and Witte JS. (1999). *Cancer Epidemiol. Biomarkers Prev.*, **8**, 901–905.
- Platz EA, Rimm EB, Willett WC, Kantoff PW and Giovannucci E. (2000). *J. Natl. Cancer Inst.*, **92**, 2009–2017.
- Powell IJ. (1998). *Semin. Urol. Oncol.*, **16**, 221–226.
- Rubin MA, Zhou M, Dhanasekaran SM, Varambally S, Barrette TR, Sanda MG, Pienta KJ, Ghosh D and Chinnaiyan AM. (2002). *JAMA*, **287**, 1662–1670.
- Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, Tamayo P, Renshaw AA, D'Amico AV, Richie JP, Lander ES, Loda M, Kantoff PW, Golub TR and Sellers WR. (2002). *Cancer Cell*, **1**, 203–209.
- Small EJ. (1998). *Curr. Opin. Oncol.*, **10**, 244–252.
- Srikantan V, Zou Z, Petrovics G, Xu LL, Augustus M, Davis L, Livezey JR, Connell T, Sesterhenn IA, Yoshino K, Buzard GS, Mostofi FK, McLeod DG, Moul JW and Srivastava S. (2000). *Proc. Natl. Acad. Sci. USA*, **97**, 12216–12221.
- Szymanski M and Barciszewski J. (2002). *Genome Biol.*, **3**, 51–58.
- Tam W, Ben-Yehuda D and Hayward WS. (1997). *Mol. Cell. Biol.*, **17**, 1490–1502.

- Tam W, Hughes SH, Hayward WS and Besmer PJ. (2002). *Virology*, **76**, 4275–4286.
- Taylor JA, Hirvonen A, Watson M, Pittman G, Mohler JL and Bell DA. (1996). *Cancer Res.*, **56**, 4108–4110.
- Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA and Chinnaiyan AM. (2002). *Nature*, **419**, 624–629.
- Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson Jr HF and Hampton GM. (2001). *Cancer Res.*, **61**, 5974–5978.
- Xu J, Zheng SL, Komiya A, Mychaleckyj JC, Isaacs SD, Hu JJ, Sterling D, Lange EM, Hawkins GA, Turner A, Ewing CM, Faith DA, Johnson JR, Suzuki H, Bujnovszky P, Wiley KE, DeMarzo AM, Bova GS, Chang B, Hall MC, McCullough DL, Partin AW, Kassabian VS, Carpten D, Bailey-Wilson JE, Trent JM, Ohar J, Bleecker ER, Walsh PC, Isaacs WB and Meyers DA. (2002). *Nat. Genet.*, **32**, 321–325.
- Xu LL, Srikantan V, Sesterhenn IA, Augustus M, Dean R, Moul JW, Carter KC and Srivastava S. (2000). *J. Urol.*, **163**, 972–979.
- Yan H, Dobbie Z, Gruber SB, Markowitz S, Romans K, Giardiello FM, Kinzler KW and Vogelstein B. (2002). *Nat. Genet.*, **30**, 25–26.
- Yang G, Addai J, Ittmann M, Wheeler TM and Thompson TC. (2000). *Clin. Cancer Res.*, **6**, 3430–3433.

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☐ 1: [AF223389](#). Homo sapiens PCGE...[gi:11066459]

LOCUS AF223389 1603 bp mRNA linear PRI 01-DEC-2000

DEFINITION Homo sapiens PCGEM1 gene, non-coding mRNA.

ACCESSION AF223389

VERSION AF223389.1 GI:11066459

KEYWORDS

SOURCE Homo sapiens (human)

ORGANISM Homo sapiens

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 1603)

AUTHORS Srikantan,V., Zou,Z., Petrovics,G., Xu,L., Augustus,M., Davis,L., Livezey,J.R., Connell,T., Sesterhenn,I.A., Yoshino,K., Buzard,G.S., Mostofi,F.K., McLeod,D.G., Moul,J.W. and Srivastava,S.

TITLE PCGEM1, a prostate-specific gene, is overexpressed in prostate cancer

JOURNAL Proc. Natl. Acad. Sci. U.S.A. 97 (22), 12216-12221 (2000)

MEDLINE 20504490

PUBMED 11050243

REFERENCE 2 (bases 1 to 1603)

AUTHORS Srikantan,V., Zou,Z., Xu,L., Petrovics,G., Augustus,M., Davis,L., Livezey,J.R., Connell,T., Sesterhenn,I.A., Yoshino,K., Buzard,G.S., Mostofi,F.K., McLeod,D.G., Moul,J.W. and Srivastava,S.

TITLE Direct Submission

JOURNAL Submitted (11-JAN-2000) Center for Prostate Disease Research, 1530 East Jefferson Street, Rockville, MD 20852, USA

FEATURES Location/Qualifiers

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 /note="detectable only in prostate; expression regulated by androgen; has tumor-associated over-expression"

ORIGIN

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